IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of Haaning, et al.

Serial No.: 10/529,624 Group Art Unit: 1652

Filed: October 13, 2005 Examiner: Hope A. Robinson

For: Factor VII or VIIa Variants Having Increased Clotting Activity

CERTIFICATION OF MAILING BY EFS-WEB FILING

I hereby certify that this correspondence and any papers referred to as attached are being electronically transmitted to the U.S. Patent and Trademark Office through the EFS.

Date: February 2, 2011

/ Fernando I. Santos /

Fernando I. Santos

REPLY AND AMENDMENT

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

In reply to the Final Office Action mailed October 13, 2010, Applicants respectfully request reconsideration and reexamination of the present application in view of the following remarks.

Applicants respectfully petition the Commissioner for Patents under the provisions of 37 C.F.R. 1.136(a) to extend the time for filing a response to the Office Action for one (1) month from January 13, 2011 to February 13, 2011. Please charge the fee for the one-month extension of time to Deposit Account No. 03-4000.

AMENDMENTS

IN THE SPECIFICATION:

Kindly amend the specification as shown in the attached sheets (pages 4-14).

IN THE CLAIMS:

Kindly amend the claims as follows:

Please amend claims 1 and 2, as shown in the attached sheets (pages 15-17).

REMARKS

Applicants respectfully request reconsideration and reexamination of the present application in light of the amendments and remarks below.

Claims 1, 2, 7, 13, 14, 25-28, 30, 32 35, 50, 51, 53-55, 62, and 63 are pending in this application. Claims 51, 53-54, and 62-63 have been withdrawn from consideration.

Applicants would like to thank Examiner Robinson for taking the time to discuss the present application by phone. Applicants noted during the call that an Amendment was filed on December 19, 2008. Examiner Robinson believes that those amendments were not entered due to misannotation of that reply. Examiner Robison suggested resubmitting the same amendments in the present reply. Accordingly, Applicants have resubmitted amendments made to the Specification and to the Claims.

Claims 1 and 2 have been amended. Support for the amended claim may be found, for example, on pages 19 and 20 of the specification. These claim amendments are made to clarify the subject matter therein. These amendments are submitted in order to place the claims in condition for allowance, and do not disclaim any subject matter to which the Applicants are entitled.

Rejection Under 35 U.S.C. § 112, first paragraph

Examiner has maintained the rejection to claims 1, 2, 7, 13, 14, 25-28, 30, 32 35, 50 and 55 under 35 U.S.C. § 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention.

Applicants have amended the claims 1 and 2 to remove the language cited by Examiner, "in no more than 15 amino acid residues." As presently presented, claim 1 provides a Factor VII or Factor VIIa polypeptide variant having the modification L65Q.

It is thus submitted that the claims meet the requirements of 35 USC § 112, first paragraph, and reconsideration and withdrawal of the present rejection is respectfully requested.

CONCLUSION

For the foregoing reasons, Applicants submit that the claims are in condition for allowance and Applicants respectfully request reexamination of the present application, reconsideration and withdrawal of the present rejections and objections, and entry of the amendments. Should there be any further matter requiring consideration, Examiner Robinson is invited to contact the undersigned counsel.

If there are any further fees due in connection with the filing of the present reply, please charge the fees to undersigned's Deposit Account No. 03-4000. If a fee is required for an extension of time not accounted for, such an extension is requested and the fee should also be charged to undersigned's deposit account.

Respectfully submitted,

/ Fernando I. Santos /

Date: February 2, 2011

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AMENDMENTS TO THE SPECIFICATION

1) Please insert the following paragraph beginning at page 1, line 1:

This application is the national stage of International Patent Application No. PCT/DK2003/000632, filed on September 26, 2003, which claims the benefit of U.S. Provisional Patent Application Serial No. 60/479,642, filed on June 19, 2003 and U.S. Provisional Patent Application Serial No. 60/414,836, filed on September 30, 2002, the disclosures of each of-which are incorporated by reference herein in their entirety for all purposes.

2) The paragraph beginning at page 22, line 1 should be replaced with the following replacement paragraph:

With markings:

In order to avoid to much disruption of the structure and function of the FVII or FVIIa polypeptide, the FVII or FVIIa polypeptide variant of the invention typically comprises an amino acid sequence having at least 95% identity with SEQ ID NO:1, such as at least 96% identity with SEQ ID NO:1, e.g. at least 97% identity with SEQ ID NO:1, at least 98% identity with SEQ ID NO:1, or at least 99% identity with SEQ ID NO:1. Amino acid sequence identity is conveniently determined from aligned sequences, using e.g. the ClustalW program, version 1.8, June 1999, using default parameters (Thompson et al., 1994, ClustalW: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice, Nucleic Acids Research, 22: 4673-4680) or from the PFAM families database version 4. 0 (~~http://~~pfam.wustl.edu/) *(Nucleic Acids Res*. 1999 Jan 1; 27(1):260-2) by use of GENEDOC version 2.5 (Nicholas, K.B., Nicholas H.B. Jr., and Deerfield, D.W. II. 1997 GeneDoc: Analysis and Visualization of Genetic Variation, EMBNEW.NEWS 4: 14; Nicholas, K.B. and Nicholas H.B. Jr. 1997 GeneDoc: Analysis and Visualization of Genetic Variation).

Without markings:

In order to avoid to much disruption of the structure and function of the FVII or FVIIa polypeptide, the FVII or FVIIa polypeptide variant of the invention typically comprises an amino acid sequence having at least 95% identity with SEQ ID NO:1, such as at least 96% identity with SEQ ID NO:1, e.g. at least 97% identity with SEQ ID NO:1, at least 98% identity with SEQ ID NO:1, or at least 99% identity with SEQ ID NO:1. Amino acid sequence identity is conveniently determined from aligned

sequences, using e.g. the ClustalW program, version 1.8, June 1999, using default parameters (Thompson et al., 1994, ClustalW: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice, Nucleic Acids Research, 22: 4673-4680) or from the PFAM families database version 4. 0 (pfam.wustl.edu/) *(Nucleic Acids Res.* 1999 Jan 1; 27(1):260-2) by use of GENEDOC version 2.5 (Nicholas, K.B., Nicholas H.B. Jr., and Deerfield, D.W. II. 1997 GeneDoc: Analysis and Visualization of Genetic Variation, EMBNEW.NEWS 4: 14; Nicholas, K.B. and Nicholas H.B. Jr. 1997 GeneDoc: Analysis and Visualization of Genetic Variation).

3) The paragraph beginning at page 48, line 1 should be replaced with the following replacement paragraph:

With markings:

Examples of suitable yeast host cells include strains of *Saccharomyces*, e.g. S. *cerevisiae*, *Schizosaccharomyces*, *Kluyveromyces*, *Pichia*, such as P. *pastoris* or P. methanolica, *Hansenula*, such as *H. Polymorpha* or *Yarrowia*. Yeast may be transformed using the procedures described by Becker and Guarente, *In* Abelson, J.N. and Simon, M.I., editors, *Guide to Yeast Genetics and Molecular Biology, Methods in Enzymology*, Volume 194, pp 182-187, Academic Press, Inc., New York; Ito *et al.*, 1983, *Journal of Bacteriology* 153: 163; Hinnen *et al.*, 1978, *Proceedings of the National Academy of Sciences USA* 75: 1920: and as disclosed by Clontech Laboratories, Inc, Palo Alto, CA, USA (in the product protocol for the Yeastmaker™ Yeast Transformation System Kit). Examples of suitable insect host cells include a *Lepidoptora* cell line, such as *Spodoptera frugiperda* (Sf9 or Sf21) or *Trichoplusioa ni* cells (High Five) (US 5,077, 214). Transformation of insect cells and production of heterologous polypeptides therein may be performed as described by Invitrogen. Examples of suitable mammalian host cells include Chinese hamster ovary (CHO) cell lines, (e.g. CHO-K1; ATCC CCL-61), Green Monkey cell lines (COS) (e.g. COS 1 (ATCC CRL-1650), COS 7 (ATCC CRL-1651)); mouse s cells (e.g. NS/O), Baby Hamster Kidney (BHK) cell lines (e.g. ATCC CRL-1632 or ATCC CCL-10), and human cells (e.g. HEK 293 (ATCC CRL-1573)), as well as plant cells in tissue culture. Additional suitable cell lines are known in the art and available from public depositories such as the American Type Culture Collection, ~~Rockville, Maryland~~ Manassas, VA. Also, the mammalian cell, such as a CHO cell, may be modified to express sialyltransferase, e.g. 1,6-sialyltransferase, e.g. as described in US 5,047,335, in order to provide improved glycosylation of the polypeptide variant.

Without markings:

Examples of suitable yeast host cells include strains of *Saccharomyces*, e.g. S. *cerevisiae*, *Schizosaccharomyces*, *Kluyveromyces*, *Pichia*, such as P. *pastoris* or P. methanolica, *Hansenula*, such as *H. Polymorpha* or *Yarrowia*. Yeast may be transformed using the procedures described by Becker and Guarente, *In* Abelson, J.N. and Simon, M.I., editors, *Guide to Yeast Genetics and Molecular Biology, Methods in Enzymology*, Volume 194, pp 182-187, Academic Press, Inc., New York; Ito *et al.*, 1983, *Journal of Bacteriology* 153: 163; Hinnen *et al.*, 1978, *Proceedings of the National Academy of Sciences USA* 75: 1920: and as disclosed by Clontech Laboratories, Inc, Palo Alto, CA, USA (in the product protocol for the Yeastmaker™ Yeast Transformation System Kit). Examples of suitable insect host cells include a *Lepidoptora* cell line, such as *Spodoptera frugiperda* (Sf9 or Sf21) or *Trichoplusioa ni* cells (High Five) (US 5,077, 214). Transformation of insect cells and production of heterologous polypeptides therein may be performed as described by Invitrogen. Examples of suitable mammalian host cells include Chinese hamster ovary (CHO) cell lines, (e.g. CHO-K1; ATCC CCL-61), Green Monkey cell lines (COS) (e.g. COS 1 (ATCC CRL-1650), COS 7 (ATCC CRL-1651)); mouse s cells (e.g. NS/O), Baby Hamster Kidney (BHK) cell lines (e.g. ATCC CRL-1632 or ATCC CCL-10), and human cells (e.g. HEK 293 (ATCC CRL-1573)), as well as plant cells in tissue culture. Additional suitable cell lines are known in the art and available from public depositories such as the American Type Culture Collection, Manassas, VA. Also, the mammalian cell, such as a CHO cell, may be modified to express sialyltransferase, e.g. 1,6-sialyltransferase, e.g. as described in US 5,047,335, in order to provide improved glycosylation of the polypeptide variant.

4) The paragraph beginning at page 48, line 26 should be replaced with the following replacement paragraph:

With markings:

Methods for introducing exogeneous DNA into mammalian host cells include calcium phosphate-mediated transfection, electroporation, DEAE-dextran mediated transfection, liposome-mediated transfection, viral vectors and the transfection method described by Life Technologies Lt,. Paisley, UK using ~~Lipofectamine~~ Lipofectamine™ 2000. These methods are well known in the art and e.g. described by Ausbel et al. (eds.), 1996, Current Protocols in Molecular Biology, John Wiley & Sons, New York, USA. The cultivation of mammalian cells are conducted according to established methods, e.g. as disclosed in Animal Cell Biotechnology, Methods and Protocols, Edited by Nigel Jenkins, 1999, Human Press Inc, Totowa, New Jersey, USA and Harrison MA and Rae IF, General Techniques of Cell Culture, Cambridge University Press 1997.

Without markings:

Methods for introducing exogeneous DNA into mammalian host cells include calcium phosphate-mediated transfection, electroporation, DEAE-dextran mediated transfection, liposome-mediated transfection, viral vectors and the transfection method described by Life Technologies Lt,. Paisley, UK using Lipofectamine™ 2000. These methods are well known in the art and e.g. described by Ausbel et al. (eds.), 1996, Current Protocols in Molecular Biology, John Wiley & Sons, New York, USA. The cultivation of mammalian cells are conducted according to established methods, e.g. as disclosed in Animal Cell Biotechnology, Methods and Protocols, Edited by Nigel Jenkins, 1999, Human Press Inc, Totowa, New Jersey, USA and Harrison MA and Rae IF, General Techniques of Cell Culture, Cambridge University Press 1997.

5) The paragraph beginning at page 56, line 23 should be replaced with the following replacement paragraph:

With markings:

Non-ionic surfactants or detergents (also known as "wetting agents") may be present to help solubilizing the therapeutic agent as well as to protect the therapeutic polypeptide against agitation-induced aggregation, which also permits the formulation to be exposed to shear surface stress without causing denaturation of the polypeptide. Suitable non-ionic surfactants include polysorbates (20, 80, etc.), polyoxamers (184, 188 etc.), Pluronic® polyols, polyoxyethylene sorbitan monoethers (Tween®-20 (polyoxyethylenesorbitan monolaurate), Tween®-80 (polyoxyethylenesorbitan monooleate), etc.).

Without markings:

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6) The paragraph beginning at page 60, line 31 should be replaced with the following replacement paragraph:

With markings:

The clotting activity of the FVIIa and variants thereof were measured in one-stage assays and the clotting times were recorded on a Thrombotrack® IV coagulometer (Medinor). Factor VII-depleted human plasma (American Diagnostica) was reconstituted and equilibrated at room temperature for 15-20 minutes. 50 microliters of plasma was then transferred to the coagulometer cups.

Without markings:

The clotting activity of the FVIIa and variants thereof were measured in one-stage assays and the clotting times were recorded on a Thrombotrack® IV coagulometer (Medinor). Factor VII-depleted human plasma (American Diagnostica) was reconstituted and equilibrated at room temperature for 15-20 minutes. 50 microliters of plasma was then transferred to the coagulometer cups.

7) The paragraph beginning at page 61, line 8 should be replaced with the following replacement paragraph:

With markings:

To measure the clotting activity in the absence of TF the same assay was used without addition of thromboplastin. Data was analysed using PRISM® software.

Without markings:

To measure the clotting activity in the absence of TF the same assay was used without addition of thromboplastin. Data was analysed using PRISM® software.

8) The paragraph beginning at page 61, line 12 should be replaced with the following replacement paragraph:

With markings:

The clotting activity of the FVIIa and variants thereof were measured in one-stage assays and the clotting times were recorded on a Thrombotrack® IV coagulometer (Medinor). 100 µl of FVIIa or variants thereof were diluted in a buffer containing 10 mM glycylglycine, 50 mM NaCl, 37.5 mM $CaCl_2$, pH 7.35 and transferred to a reaction cup. The clotting reaction was initiated by addition of 50 µl blood

containing 10% 0.13 M tri-sodium citrate as anticoagulant. Data was analyzed using Excel® or PRISM® software.

Without markings:

The clotting activity of the FVIIa and variants thereof were measured in one-stage assays and the clotting times were recorded on a Thrombotrack® IV coagulometer (Medinor). 100 µl of FVIIa or variants thereof were diluted in a buffer containing 10 mM glycylglycine, 50 mM NaCl, 37.5 mM $CaCl_2$, pH 7.35 and transferred to a reaction cup. The clotting reaction was initiated by addition of 50 µl blood containing 10% 0.13 M tri-sodium citrate as anticoagulant. Data was analyzed using Excel® or PRISM® software.

9) The paragraph beginning at page 61, line 30 should be replaced with the following replacement paragraph:

With markings:

FVII/FVIIa (or variant) concentrations are determined by ELISA. Wells of a microtiter plate are coated with an antibody directed against the protease domain using a solution of 2 µg/ml in PBS (100 µl per well). After overnight coating at R.T., the wells are washed 4 times with THT buffer (100 mM NaCl, 50 mM Tris-HCl pH 7.2 0.05% Tween®-20 (polyoxyethylenesorbitan monolaurate)). Subsequently, 200 µl of 1% Casein (diluted from 2.5% stock using 100 mM NaCl, 50 mM Tris-HCl pH 7.2) is added per well for blocking. After 1 hr incubation at R.T., the wells are emptied, and 100 µl of sample (optionally diluted in dilution buffer (THT + 0.1% Casein)) is added. After another incubation of 1 hr at room temperature, the wells are washed 4 times with THT buffer, and 100 µl of a biotin-labelled antibody directed against the EGF-like domain (1 µg/ml) is added. After another 1 hr incubation at R.T. , followed by 4 more washes with THT buffer, 100 µl of streptavidin-horse radish peroxidase (DAKO A/S, Glostrup, Denmark, 1/10000 diluted) is added. After another 1 hr incubation at R.T., followed by 4 more washes with THT buffer, 100 µl of TMB (3,3',5,5'-tetramethylbenzidine, Kem-en-Tech A/S, Denmark) is added. After 30 min incubation at R.T. in the dark, 100 µl of 1 M H2SO4 is added and OD_{450nm} is determined. A standard curve is prepared using rhFVIIa (NovoSeven®).

Without markings:

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per well). After overnight coating at R.T., the wells are washed 4 times with THT buffer (100 mM NaCl, 50 mM Tris-HCl pH 7.2 0.05% Tween®-20 (polyoxyethylenesorbitan monolaurate)). Subsequently, 200 µl of 1% Casein (diluted from 2.5% stock using 100 mM NaCl, 50 mM Tris-HCl pH 7.2) is added per well for blocking. After 1 hr incubation at R.T., the wells are emptied, and 100 µl of sample (optionally diluted in dilution buffer (THT + 0.1% Casein)) is added. After another incubation of 1 hr at room temperature, the wells are washed 4 times with THT buffer, and 100 µl of a biotin-labelled antibody directed against the EGF-like domain (1 µg/ml) is added. After another 1 hr incubation at R.T. , followed by 4 more washes with THT buffer, 100 µl of streptavidin-horse radish peroxidase (DAKO A/S, Glostrup, Denmark, 1/10000 diluted) is added. After another 1 hr incubation at R.T., followed by 4 more washes with THT buffer, 100 µl of TMB (3,3',5,5'-tetramethylbenzidine, Kem-en-Tech A/S, Denmark) is added. After 30 min incubation at R.T. in the dark, 100 µl of 1 M H2SO4 is added and OD_{450nm} is determined. A standard curve is prepared using rhFVIIa (NovoSeven®).

10) The paragraph beginning at page 68, line 12 should be replaced with the following replacement paragraph:

With markings:

The cell line CHO K1 (ATCC # CCL-61) is seeded at 50% confluence in T-25 flasks using MEMα, 10% FCS (Gibco/BRL, Cat # 10091), P/S and 5 µg/ml phylloquinone and allowed to grow until confluent. The confluent mono cell layer is transfected with 5 µg of the relevant plasmid described above using the ~~Lipofectamine~~ Lipofectamine™ 2000 transfection agent (Life ~~technologies~~ Technologies) according to the manufacturer's instructions. Twenty four hours post transfection a sample is drawn and quantified using *e.g.* an ELISA recognizing the EGF1 domain of hFVII. At this time point relevant selection (e.g. Hygromycin B) may be applied to the cells with the purpose of generating a pool of stable transfectants. When using CHO K1 cells and the Hygromycin B resistance gene as selectable marker on the plasmid, this is usually achieved within one week.

Without markings:

The cell line CHO K1 (ATCC # CCL-61) is seeded at 50% confluence in T-25 flasks using MEMα, 10% FCS (Gibco/BRL, Cat # 10091), P/S and 5 µg/ml phylloquinone and allowed to grow until confluent. The confluent mono cell layer is transfected with 5 µg of the relevant plasmid described above using the Lipofectamine™ 2000 transfection agent (Life Technologies) according to the manufacturer's instructions. Twenty four hours post transfection

a sample is drawn and quantified using *e.g.* an ELISA recognizing the EGF1 domain of hFVII. At this time point relevant selection (e.g. Hygromycin B) may be applied to the cells with the purpose of generating a pool of stable transfectants. When using CHO K1 cells and the Hygromycin B resistance gene as selectable marker on the plasmid, this is usually achieved within one week.

11) The paragraph beginning at page 69, line 17 should be replaced with the following replacement paragraph:

With markings:

The eluate from the first chromatographic step was loaded directly onto a second and final chromatographic column, which consisted of a POROS® HQ50 column pre-equilibrated with 10 mM Tris, 25 mM NaCl, 5 mM EDTA, pH 8.6. FVIIa was eluted from the POROS® HQ50 column using 10 mM Tris, 25 mM NaCl, 35 mM $CaCl_2$, pH 7.5 after washing the column with 10 mM Tris, 25 mM NaCl, pH 8.6. FVIIa eluted from the POROS® HQ50 column was stored at-80°C without further modification.

Without markings:

The eluate from the first chromatographic step was loaded directly onto a second and final chromatographic column, which consisted of a POROS® HQ50 column pre-equilibrated with 10 mM Tris, 25 mM NaCl, 5 mM EDTA, pH 8.6. FVIIa was eluted from the POROS® HQ50 column using 10 mM Tris, 25 mM NaCl, 35 mM $CaCl_2$, pH 7.5 after washing the column with 10 mM Tris, 25 mM NaCl, pH 8.6. FVIIa eluted from the POROS® HQ50 column was stored at-80°C without further modification.

12) The paragraph beginning at page 69, line 26 should be replaced with the following replacement paragraph:

With markings:

FVII and FVII variants are purified as follows: The procedure is performed at 4°C. The harvested culture media from large-scale production is ultrafiltered using a Millipore TFF system with 30 KDa cut-off Pellicon membranes. After concentration of the medium, citrate is added to 5 mM and the pH is adjusted to 8.6. If necessary, the conductivity is lowered to below 10 mS/cm. Subsequently, the sample is applied to a ~~Q-sepharose~~ Q-Sepharose™ FF column, equilibrated with 50 mM NaCl, 10 mM Tris pH 8.6. After washing the column with 100 mM NaCl, 10 mM Tris pH 8.6, followed by 150 mM NaCl, 10 mM Tris pH 8.6, FVII is eluted using 10 mM Tris, 25 mM NaCl, 35 mM $CaCl_2$, pH 8.6.

Without markings:

FVII and FVII variants are purified as follows: The procedure is performed at 4°C. The harvested culture media from large-scale production is ultrafiltered using a Millipore TFF system with 30 KDa cut-off Pellicon membranes. After concentration of the medium, citrate is added to 5 mM and the pH is adjusted to 8.6. If necessary, the conductivity is lowered to below 10 mS/cm. Subsequently, the sample is applied to a Q-Sepharose™ FF column, equilibrated with 50 mM NaCl, 10 mM Tris pH 8.6. After washing the column with 100 mM NaCl, 10 mM Tris pH 8.6, followed by 150 mM NaCl, 10 mM Tris pH 8.6, FVII is eluted using 10 mM Tris, 25 mM NaCl, 35 mM $CaCl_2$, pH 8.6.

13) The paragraph beginning at page 70, line 1 should be replaced with the following replacement paragraph:

With markings:

For the second chromatographic step, an affinity column is prepared by coupling of a monoclonal Calcium-dependent antiGla-domain antibody to CNBr-activated Sepharose™ FF. About 5.5 mg antibody is coupled per ml resin. The column is equilibrated with 10 mM Tris, up to 100 mM NaCl, 35 mM $CaCl_2$, pH 7.5. NaCl is added to the sample to a concentration of 100 mM NaCl and the pH is adjusted to 7. 4-7.6. After O/N application of the sample, the column is washed with up to 100 mM NaCl, 35 mM $CaCl_2$, 10 mM Tris pH 7.5, and the FVII protein is eluted with 100 mM NaCl, 50 mM citrate, 75 mM Tris pH 7.5.

Without markings:

For the second chromatographic step, an affinity column is prepared by coupling of a monoclonal Calcium-dependent antiGla-domain antibody to CNBr-activated Sepharose™ FF. About 5.5 mg antibody is coupled per ml resin. The column is equilibrated with 10 mM Tris, up to 100 mM NaCl, 35 mM $CaCl_2$, pH 7.5. NaCl is added to the sample to a concentration of 100 mM NaCl and the pH is adjusted to 7. 4-7.6. After O/N application of the sample, the column is washed with up to 100 mM NaCl, 35 mM $CaCl_2$, 10 mM Tris pH 7.5, and the FVII protein is eluted with 100 mM NaCl, 50 mM citrate, 75 mM Tris pH 7.5.

14) The paragraph beginning at page 70, line 8 should be replaced with the following replacement paragraph:

With markings:

For the third chromatographic, the conductivity of the sample is lowered to below 10 mS/cm, if necessary, and the pH is adjusted to 8.6. The sample is then applied to a ~~Q-sepharose~~ Q-Sepharose™ column (equilibrated with 50 mM NaCI, 10 mM Tris pH 8.6) at a density around 3-5 mg protein per ml gel to obtain efficient activation. After application, the column is washed with 50 mM NaCI, 10 mM Tris pH 8.6 for about 4 hours with a flow of 3-4 column volumes (cv) per hour. The FVII protein is eluted using a gradient of 0-100% of 500 mM NaCl, 10 mM Tris pH 8.6 over 40 cv. FVII containing fractions are pooled.

With markings:

For the third chromatographic, the conductivity of the sample is lowered to below 10 mS/cm, if necessary, and the pH is adjusted to 8.6. The sample is then applied to a Q-Sepharose™ column (equilibrated with 50 mM NaCI, 10 mM Tris pH 8.6) at a density around 3-5 mg protein per ml gel to obtain efficient activation. After application, the column is washed with 50 mM NaCI, 10 mM Tris pH 8.6 for about 4 hours with a flow of 3-4 column volumes (cv) per hour. The FVII protein is eluted using a gradient of 0-100% of 500 mM NaCl, 10 mM Tris pH 8.6 over 40 cv. FVII containing fractions are pooled.

15) The paragraph beginning at page 70, line 15 should be replaced with the following replacement paragraph:

With markings:

For the final chromatographic step, the conductivity is lowered to below 10 mS/cm. Subsequently, the sample is applied to a ~~Q-sepharose~~ Q-Sepharose™ column (equilibrated with 140 mM NaCl, 10 mM glycylglycine pH 8.6) at a concentration of 3-5 mg protein per ml gel. The column is then washed with 140 mM NaCl, 10 mM glycylglycine pH 8.6 and FVII is eluted with 140 mM NaCl, 15 mM $CaCl_2$, 10 mM glycylglycine pH 8.6. The eluate is diluted to 10 mM $CaCl_2$ and the pH is adjusted 6.8-7.2. Finally, ~~Tween 80~~ Tween®-80 (polyoxyethylenesorbitan monooleate) is added to 0.01% and the pH is adjusted to 5.5 for storage at-80°C.

Without markings:

For the final chromatographic step, the conductivity is lowered to below 10 mS/cm. Subsequently, the sample is applied to a Q-Sepharose™ column (equilibrated with 140 mM NaCl, 10 mM glycylglycine pH 8.6) at a concentration of 3-5 mg protein per ml gel. The column is then washed with 140 mM NaCl, 10 mM glycylglycine pH 8.6 and FVII is eluted with 140 mM NaCl, 15 mM $CaCl_2$, 10 mM glycylglycine pH 8.6. The eluate is diluted to 10 mM $CaCl_2$ and the pH is adjusted 6.8-7.2. Finally, Tween®-80 (polyoxyethylenesorbitan monooleate) is added to 0.01% and the pH is adjusted to 5.5 for storage at-80°C.

CLAIM AMENDMENTS

1. (Currently Amended) ~~An isolated or~~ A recombinant Factor VII (FVII) or Factor VIIa (FVIIa) polypeptide variant comprising an amino acid substitution ~~sequence which differs from the amino acid sequence of human Factor VII (hFVII) or human Factor VIIa (hFVIIa) shown in SEQ ID NO:1 with in no more than 15 amino acid residues~~, wherein the leucine (L) in position 65 of SEQ ID NO:1 is substituted with a glutamine (Q) in said variant sequence, and wherein amino acid positions of the variant sequence are numbered according to SEQ ID NO:1.

2. (Currently Amended) The variant according to claim 1, wherein said variant sequence further comprises at least one amino acid substitution selected from the group consisting of L39E, L39Q, L39H, I42R, S43H, S43Q, K62E, K62R, ~~L65S~~, F71D, F71Y, F71E, F71Q, F71N, E82Q, E82N, E82K, and F275H.

3-6. (Cancelled).

7. (Previously presented) The variant according to claim 2, wherein said at least one amino acid substitution is F71Y, K62E or S43Q.

8-12. (Cancelled).

13. (Previously presented) The variant according to claim 1, wherein said variant further comprises at least one amino acid substitution in the Gla domain.

14. (Previously presented) The variant according to claim 13, wherein said at least one amino acid substitution in the Gla domain is selected from the group consisting of P10, K32, D33 and A34.

15-24. (Cancelled).

25. (Previously presented) The variant according to claim 1, wherein an amino acid residue comprising an attachment group for a non-polypeptide moiety has been introduced in the variant sequence in a position located outside the Gla domain.

26. (Previously presented) The variant according to claim 25, wherein the non-polypeptide moiety is covalently attached to the attachment group.

27. (Original) The variant according to claim 26, wherein said non-polypeptide moiety is a sugar moiety.

28. (Previously presented) The variant according to claim 25, wherein said attachment group is a glycosylation site.

29. (Cancelled).

30. (Previously presented) The variant according to claim 28, wherein said glycosylation site is introduced by amino acid substitution.

31. (Cancelled).

32. (Previously presented) The variant according to claim 30, wherein said introduced glycosylation site is an N-glycosylation site.

33-34. (Cancelled).

35. (Previously presented) The variant according to claim 32, wherein said N-glycosylation site is introduced by a substitution selected from the group consisting of A51N, G58N, G48N+S60T, T106N, K109N, G124N, K143N+N145T, A175T, I205S, I205T, V253N, T267N, T267N+S269T, S314N+K316S, S314N+K316T, R315N+V317S, R315N+V317T, K316N+G318S, K316N+G318T, G318N, and D334N.

36-49. (Cancelled).

50. (Previously presented) The variant according to claim 1, wherein said variant is in its activated form.

51. (Withdrawn) A nucleotide sequence encoding the variant according to claim 1.

52. (Cancelled).

53. (Withdrawn) A host cell comprising the nucleotide sequence according to claim 51.

54. (Withdrawn) The host cell according to claim 53, wherein said host cell is a gammacarboxylating cell capable of *in vivo* glycosylation.

55. (Previously presented) A pharmaceutical composition comprising the variant of claim 1, and a pharmaceutically acceptable carrier or excipient.

56-61. (Cancelled).

62. (Withdrawn) A method for treating a mammal having a disease or a disorder wherein clot formation is desirable, comprising administering to a mammal in need thereof an effective amount of the pharmaceutical composition according to claim 55.

63. (Withdrawn) The method of claim 62, wherein said disease or disorder is selected from the group consisting of a hemorrhage, uncontrolled bleeding, cirrhosis, thrombocytopenia, and hemophilia.

64-66. (Cancelled).